Supplementary figure legends

Figure S1. Determination of purity of different phytochemicals. (**A**) Elution profiles of each phytochemical by HPLC detection. Ginsenoside Rg2 and gypenoside GXVII were assayed by the C18 column, and other phytochemicals were assessed with the TSK column. (**B**) Total carbohydrate content of different glucoconjugate fractions was determined by the Molisch test. The standards used in this assay were prepared from monosaccharides that constituted the glucoconjugate compounds to be tested according to the sugar composition.

Figure S2. Screen results for autophagy inducers from phytochemicals. (**A**) Representative images (left panel) and quantification of GFP-LC3B puncta in HeLa cells stably expressing GFP-LC3 cultured in normal or starvation medium, or treated with the indicated chemicals at 1 mg/ml (upper-right panel) or 100 μg/ml (lower-right panel) in normal medium for 3 h. Statistics compare each value to the one under the normal condition. Scale bar: 20 μm. (**B**) Quantification of percentage of GFP-LC3 HeLa cells with GFP-LC3 puncta in normal or starvation medium, or treated with the indicated chemicals in normal medium for 3 h. (**C**) Western blot detection (upper) and quantification (lower) of SQSTM1 and LC3 in HeLa cells cultured in normal or starvation medium, or in normal medium supplied with the indicated chemicals for 30 min. (**D**) Semiquantitative RT-PCR analyses of *SQSTM1* and *LC3* mRNA levels in HeLa cells treated with starvation medium or the indicated chemicals for 3 h. *GAPDH* was used as an internal control. (**E**) Quantification of GFP-LC3 puncta in muscle of GFP-LC3 transgenic mice injected with vehicle (DMSO) or 20 mg/kg of the indicated chemicals once daily for 3 d. Results represent mean ± s.e.m. (**F**) Summary of autophagy-activating ability of the plant-derived glycoconjugate compounds. Degree of autophagy-inducing capability is indicated as follows: +, low induction; ++, moderate induction; +++, high induction; +/- or -, variable or no effects on autophagy.

Figure S3. Secondary screen for toxicity of the indicated autophagy-inducing chemicals identified from the primary screen. (**A**) Cell viability was analyzed by MTT assays in both HeLa and PC12 cells treated with vehicle or the indicated chemicals for 24 h. (**B**) Cell viability was analyzed by MTT assays in HeLa or PC12 cells treated with vehicle or Rg2 for a time course of 3 h, 6 h, 12 h and 24 h. **, $P<0.01$; ***, $P<0.001$; compared with control, t test.

Figure S4. Rg2 induces bulk autophagy but not selective autophagy (mitophagy). (**A**) EC_{50} of Rg2 in autophagy induction. Quantification of GFP-LC3 puncta in GFP-LC3 HeLa cells after 3-h treatment of Rg2 at the indicated concentrations. (**B**) Representative images (upper panel) and quantification (lower panel) of GFP-LC3 puncta in HeLa cells stably expressing GFP-LC3 cultured in normal or starvation medium, or treated with Rg2 or protopanaxatriol (PPT) in normal medium for 3 h. (**C**) Rg2 does not induce mitophagy. Immunofluorescence images (upper panel) and quantification (lower panel) of HeLa cells expressing mCherry-PARK2/Parkin with mitochondria fragmentation and clearance. Cells were cultured in normal or starvation medium, or in normal medium supplied with CCCP, rapamycin or Rg2 for 16 h. N≥100 cells. Scale bar: 20 μm. Results represent mean ± s.e.m. *, $P<0.05$; ***, $P<0.001$; NS, not significant, t test.

Figure S5. Rg2 induces autophagy in multiple cell types. Western blot detection (upper) and quantification (lower) of SQSTM1 and LC3 in HepG2, Neuro2A or PC12 cells cultured in normal or starvation medium, or in normal medium supplied with Rg2 for 3 h.

Figure S6. The majority of Rg2 is not bound by plasma proteins and is freely available. (**A**) Chromatograms of 3 different concentrations of Rg2 (100, 50 or 10 μg/ml) after equilibrium dialysis for 48 h in a plasma protein binding assay. (**B**) Quantification of rat plasma protein binding fraction of Rg2 at the above 3 concentrations after dialysis for 48 h. The percentage of

Rg2 bound by plasma protein is approximately 42% at 10 μg/ml, 24% 50 μg/ml, and 15% at 100 μg/ml. N=3.

Figure S7. Pharmacokinetics of Rg2 in mouse brain. (**A**) LC-MS analysis of mouse brain plasma after continuous intraperitoneal (i.p.) injection of 20 mg/kg Rg2 for 2 weeks in the SIM mode. (**B**) Concentration of Rg2 in mouse brain over a time course of 8 h after i.p. injection. Results represent mean ± s.e.m. N=5.

Figure S8. Function of Rg2 in vivo. (**A**) Quantification of GFP-LC3 puncta in skeletal muscle or brain of GFP-LC3 transgenic mice over a time course of 24 h after injection with 20 mg/kg Rg2. (**B**) Body weight and food intake of WT mice injected with vehicle (DMSO) or Rg2 once/d for 3 d. Results represent mean ± s.e.m. N=3.

Figure S9. Rg2 treatment reduces the size of HFD-induced lipid droplets in liver. Representative electron microscopic images of liver sections from HFD-fed WT or $BCL2^{AAA}$ mice with or without Rg2 treatment for 4 months. Scale bar: 5 μm.

Figure S10. Rg2 does not reduce cell viability of HeLa cells expressing polyQ HTT, nor does it affect APP (Amyloid Precursor Protein) expression or general ambulation activity of 5XFAD mice. (**A**) MTT assays of stable HeLa cell lines expressing WT HTT25Q, the HTT65Q mutant or the HTT103Q mutant treated with tetracycline (+Tet) or Rg2 for 24 h. (**B**) Western blot detection of APP expression level in brain samples of 5XFAD mice treated with vehicle (DMSO) or Rg2 for 4 months. A WT mouse without APP transgene was used as negative control. (**C**) Open field tests were used to analyze general movement of 5XFAD mice treated with vehicle or Rg2 for 4 months. Mice were placed in the center of an arena, and the total distance traveled in the first 5

min was camera-recorded. Results represent mean ± s.e.m. *, $P<0.05$; NS, not significant, t test.

Supplementary videos

Video S1. Water maze test with visible platform of age-matched WT mice.

Video S2. Water maze test with visible platform of 5XFAD mice treated with vehicle for 16 weeks.

Video S3. Water maze test with visible platform of 5XFAD mice treated with Rg2 for 16 weeks.

Video S4. Water maze test with hidden platform of age-matched WT mice.

Video S5. Water maze test with hidden platform of 5XFAD mice treated with vehicle for 16 weeks.

Video S6. Water maze test with hidden platform on 5XFAD mice treated with Rg2 for 16 weeks.

Supplementary methods

Cell viability analysis.

HeLa and PC12 cells were treated with 1.0 mg/ml of the indicated metabolites for 24 and 48 h, or 0.1 mM of Rg2, or 0.1 mM of GXVII for 3, 6, 12 and 24 h, 100 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, 0.5 mg/ml, Sigma-Aldrich, M5655) were added to incubate for 4 h at 37°C. The formazan product was dissolved in 50 μl of 20% SDS with 0.04 mM HCl for overnight, and the absorbance was measured at 570 nm. Cell viability was expressed as the percentage of cell survival compared to control.

RT-PCR.

Semiquantitative RT-PCR was performed as previously described.[1] Briefly, RNA was extracted from HeLa cells using the RNeasy Mini Kit (QIAGEN, 74104), DNA was removed, and cDNA was synthesized using the SuperScript IV Reverse Transcriptase (ThermoFisher Scientific, 18090050), following manufacturers' instructions. PCR was performed to amplify *SQSTM1* (25 cycles) using the primers TGACTGGACCCATCTGTCTTC (sense) and CAGAGACCTGCAATTCTACGC (antisense), *LC3B* (25 cycles) using the primers AAACGGCCCTGACTGTAAACT (sense) and TTGTGTTTCTCCCGCTGTACT (antisense), and *GAPDH* (20 cycles) using the primers AATCCCATCACCATCTTCCA (sense) and GTCATCATATTTGGCAGGTT (antisense).

Mitophagy analyses.

For PARK2/Parkin-mediated mitophagy assays, HeLa cells were transfected with the mCherry-PARK2 plasmid. After 24 h, cells were treated with DMSO, CCCP, starvation medium, rapamycin or Rg2 for 24 h. Cells were then fixed, immunostained with anti-TOMM20 antibody

(Santa Cruz Biotechnology, sc11415; 1:500) to detect mitochondria, and analyzed by fluorescence microscopy.

Plasma protein binding study.

The plasma binding of Rg2 to rat plasma proteins was investigated by equilibrium dialysis, according to a reported method.[2] Dialysis membrane bags were refluxed in 0.01 M $NaHCO_3$ for 0.5 h, followed by multiple rinses with water and then the equilibrium dialysate immediately prior to dialysis. 30 ml of dialysates contained different amount of Rg2 to the concentration of 10, 50 and 100 μg/ml respectively. 0.5 ml of blank rat plasma was injected into the dialysis bag as control. Dialysis was performed by rotation at 100 rpm on a air bath oscillator, at 37°C for 48 h. Perchloric acid solution (0.1%, w/w) was used to estimate the leak of the plasma into the buffer outside. 0.1 ml of plasma or buffer was extracted by 1 ml acetic ether and centrifuged at 10000 rpm for 10 min. The organic layer was separated and evaporated at 45°C under vacuum condition. The residue was reconstituted in 100 μl methanol, and 10 μl was analyzed through HPLC system, using ACCHROM Uniary ODS C18 reversed-phase column (5 μm, 250 mm × 4.6 mm), eluted at the flow rate of 1.0 ml/min with the following gradient program, 0 to 10 min, 30% acetonitrile, 10 to 20 min, 30% to 40% acetonitrile, and 20 to 30 min, 100% acetonitrile, monitored by the absorbance at 203 nm. The drug protein binding fraction was calculated as: binding fraction (%) = (1-out-of-bag drug concentration/in-bag drug concentration) × 100%.

Pharmacokinetics study.

The pharmacokinetics study was performed similarly as previously reported.[3] Specifically, mice were injected with 20 mg/kg Rg2 for 2 weeks, and mice were sacrificed at 15 min, 30 min, 1 h, 4 h, 8 h and 24 h after last injection. Brain tissue harvested from mice were rinsed with 0.9% NaCl saline and gently blotted with absorbent paper. The tissue was weighed and homogenized with 2 volumes of saline. The homogenized samples were mixed for 10 min and centrifuged at

10000 rpm for 10 min at 4°C. The supernatant was evaporated at 45°C under vacuum conditions. The residue was redissolved in 0.1 ml of saline, extracted by 1 ml acetic ether and centrifuged at 10000 rpm for 10 min. The organic layer was separated and evaporated at 45°C under vacuum conditions. The residue was reconstituted in 150 μl methanol, and 10 μl of analytes were detected by an API2000 QTRAP mass spectrometer (AB SCIEX) equipped with negative selected ion monitoring (SIM) mode. The working parameters maintained were as follows: curtain gas, 20 psi; gas 1, 60 psi; gas 2, 70 psi; ion spray voltage, -4000 V. The chlorinated molecular ions of Rg2 at m/z 783.5 was monitored.

Open field testing.

5XFAD and WT mice were treated with Rg2 or DMSO (vehicle) the same as for Morris water maze analyses. The apparatus was composed of a 54.5 cm x 54.5 cm x 30.4 cm plastic box. Mice were placed into the center of the test chamber and allowed to explore for 5 minutes. The result was presented as the total distance travelled.

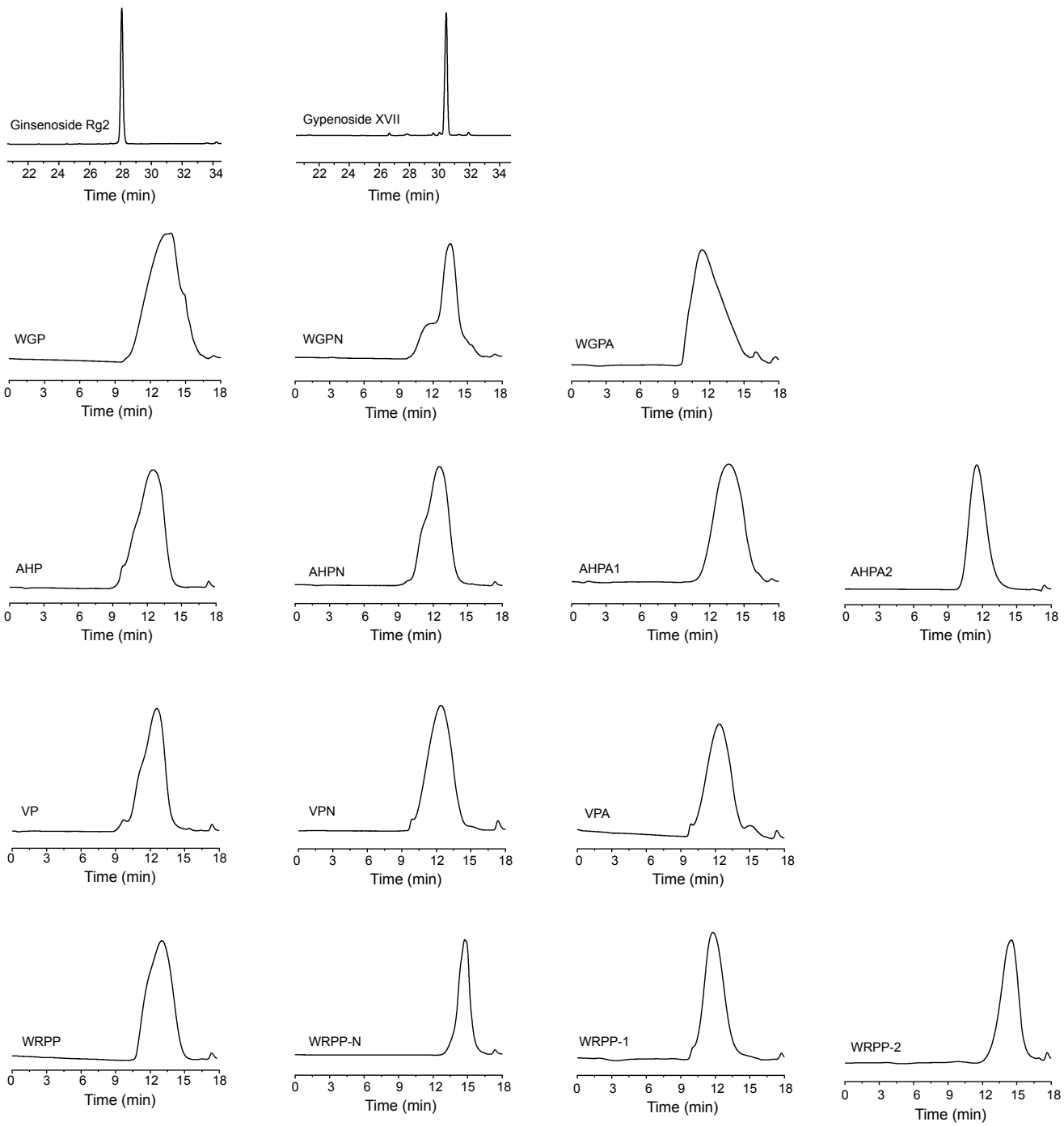
Supplementary references

1. He C, Wei Y, Sun K, Li B, Dong X, Zou Z, et al. Beclin 2 functions in autophagy, degradation of g protein-coupled receptors, and metabolism. Cell 2013; 154:1085-99.

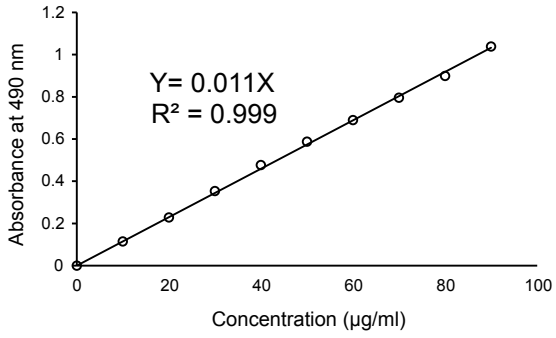
2. Gu Y, Wang G, Sun J, Jia Y, Xu M, Wang W. In vitro assessment of plasma protein binding of 20(R)-ginsenoside Rh2 by equilibrium dialysis and LC-MS analysis: a case of species differences. Biol Pharm Bull 2006; 29:951-6.

3. Feng L, Wang L, Hu C, Jiang X. Pharmacokinetics, tissue distribution, metabolism, and excretion of ginsenoside Rg1 in rats. Arch Pharm Res 2010; 33:1975-84.

A

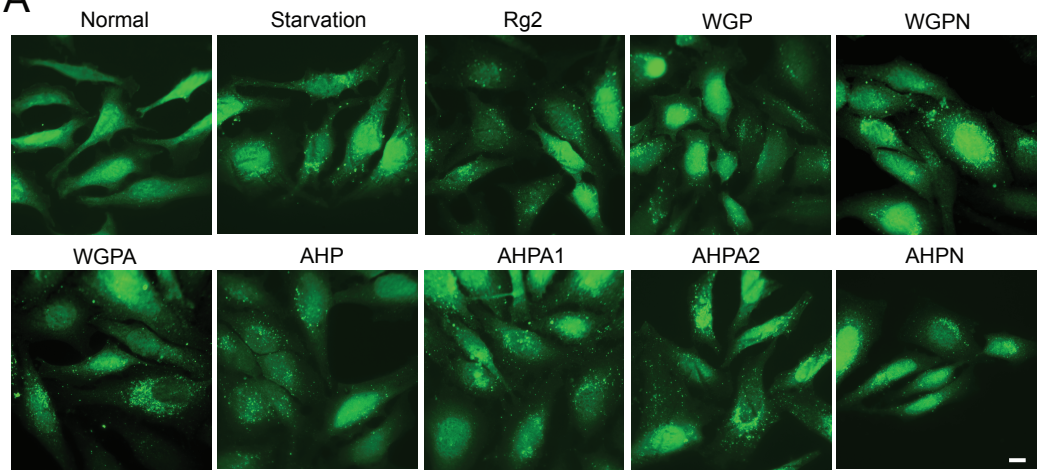


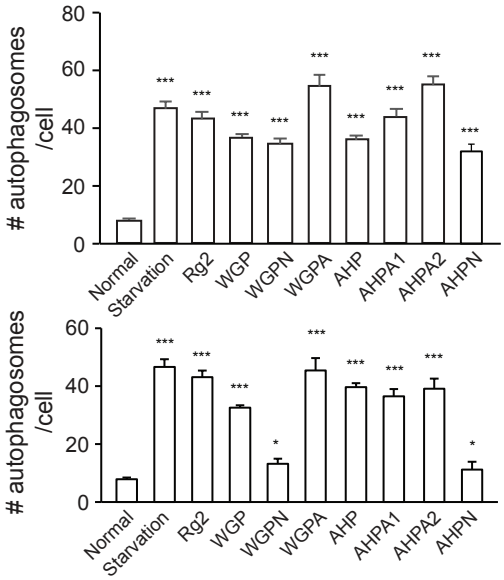
B



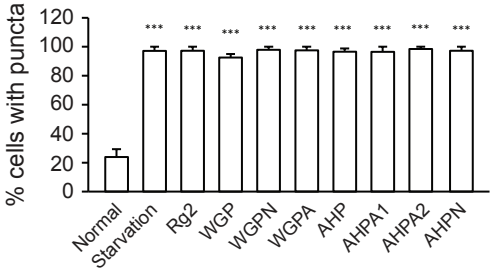
Sample	A490 nm	Purity (%)		Sample	A490 nm	Purity (%)
WGP	0.641	97.1		VP	0.639	96.8
WGP-N	0.638	96.7		VPA	0.628	95.2
WGPA	0.634	96.1		VPN	0.632	95.8
AHP	0.635	96.2		WRPP	0.63	95.5
AHPA1	0.631	95.6		WRPP-N	0.638	96.7
AHPA2	0.633	95.9		WRPP-1	0.632	95.8
AHPN	0.628	95.2		WRPP-2	0.641	97.1

A

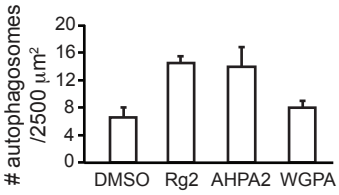




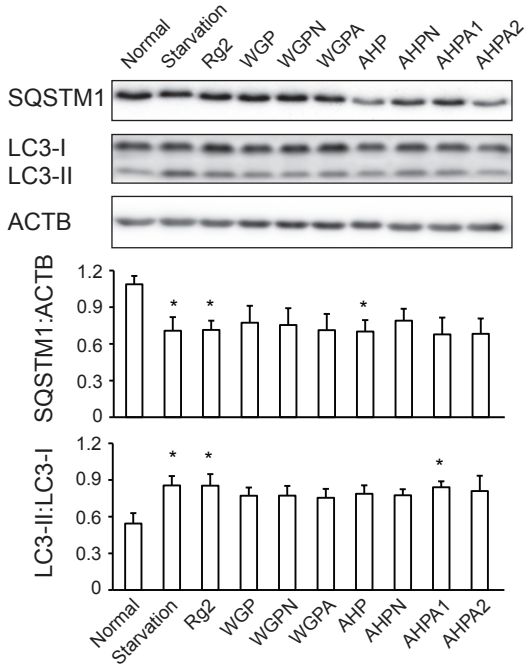
B



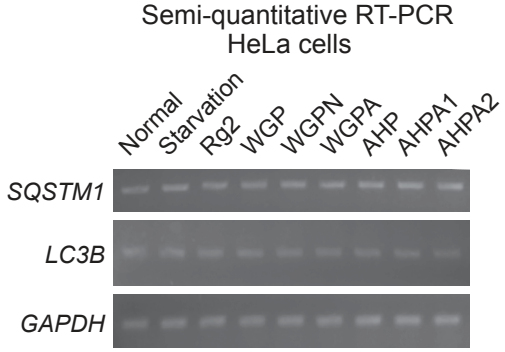
E



C



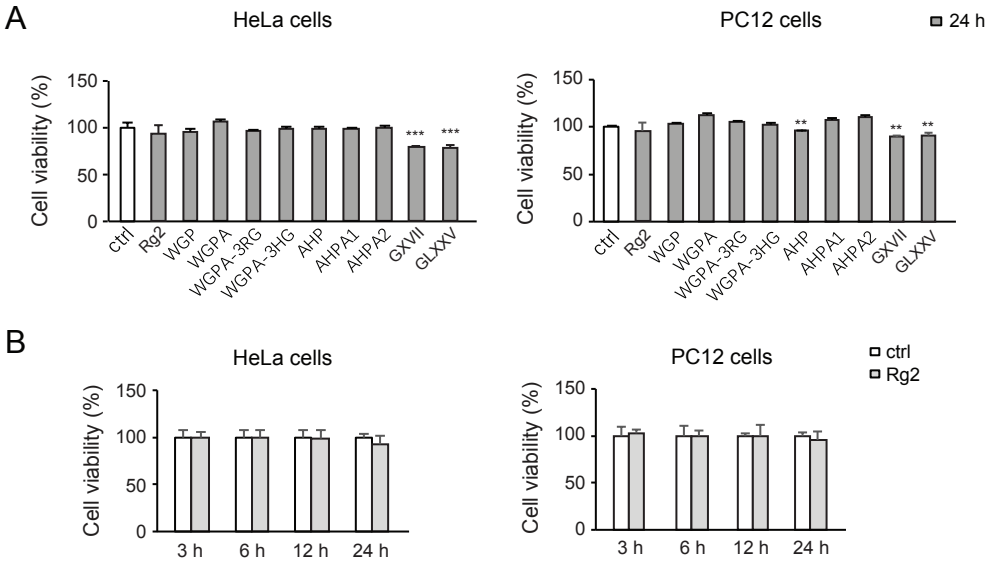
D

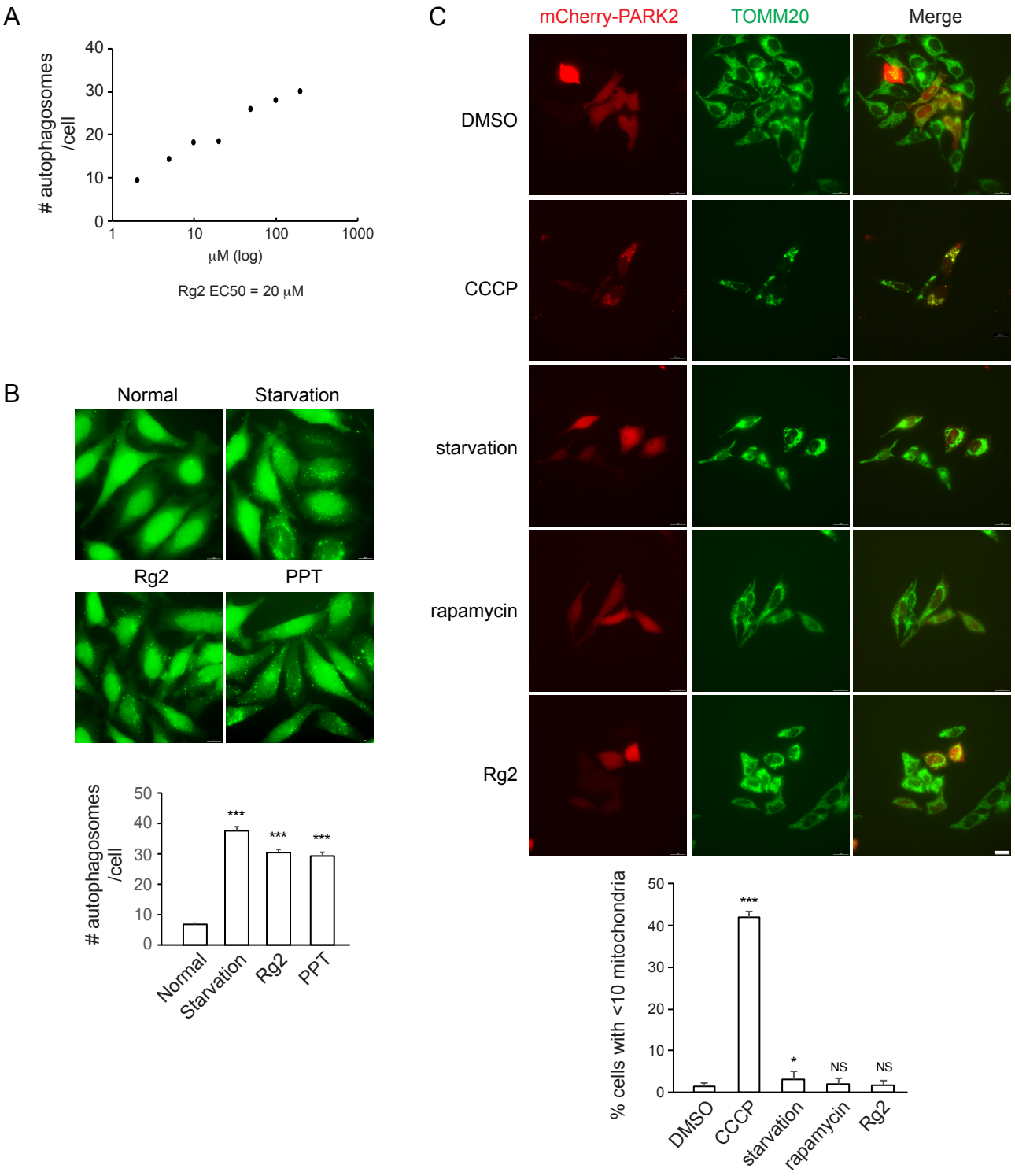


F

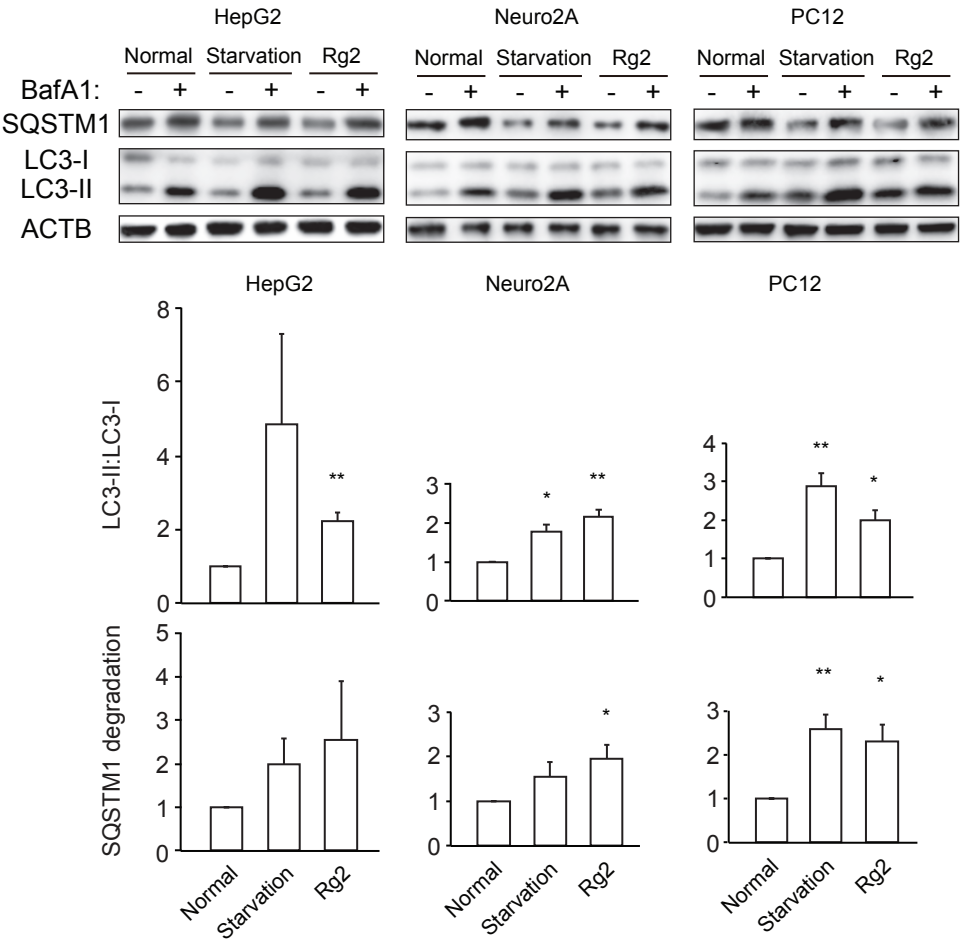
chemical	autophagy induction
Rg2	++
WGP	+
WGPN	+/-
WGPA	+++
AHP	+
AHPA1	++
AHPA2	+++
AHPN	+/-
VP	-
VPN	-
VPA	-
WRPP	-
WRPP-N	-
WRPP-1	-
WRPP-2	-
WGPA-3RG	+/-
WGPA-3HG	+/-
GXVII	-
GLXXV	+/-

Supplementary Figure 3

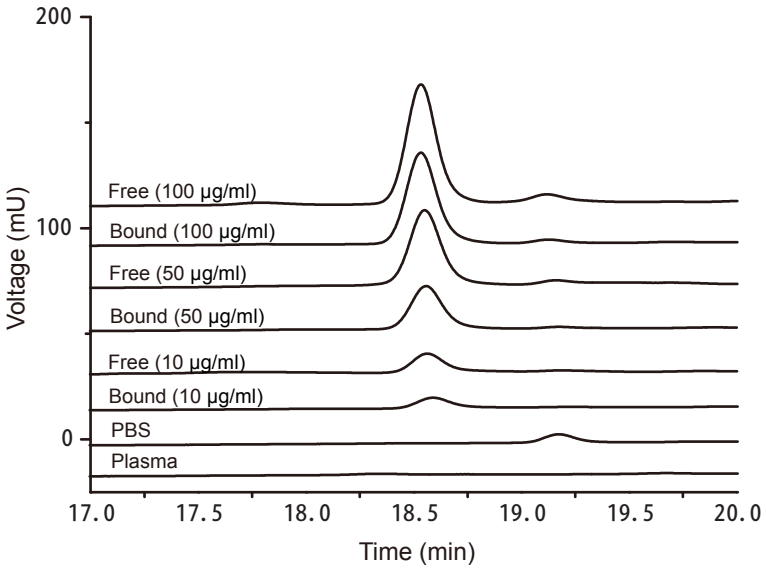



A
autophagosomes /cell
40
30
20
10
0
1
10
100
1000
μM (log)
Rg2 EC50 = 20 μM
B
Normal
Starvation
Rg2
PPT
autophagosomes /cell
50
40
30
20
10
0
***
***
***
Normal
Starvation
Rg2
PPT
C
mCherry-PARK2
TOMM20
Merge
DMSO
CCCP
starvation
rapamycin
Rg2
% cells with <10 mitochondria
50
40
30
20
10
0
***
*
NS
NS
DMSO
CCCP
starvation
rapamycin
Rg2

Supplementary Figure 5



A

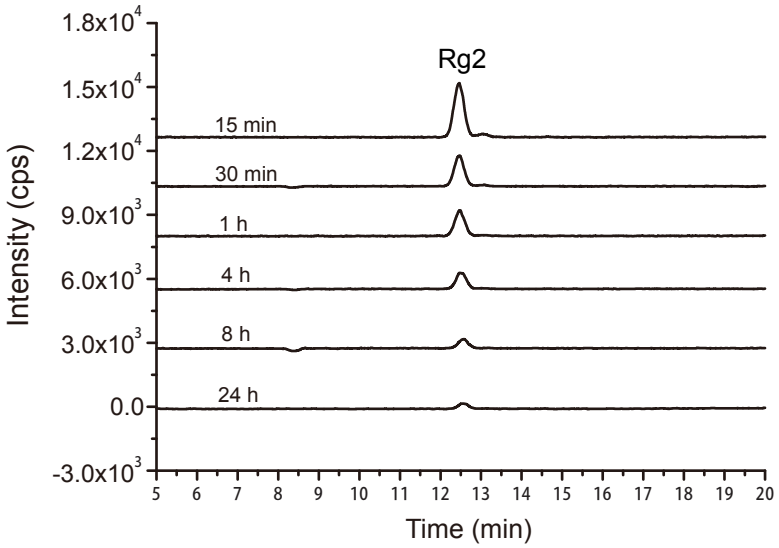


B

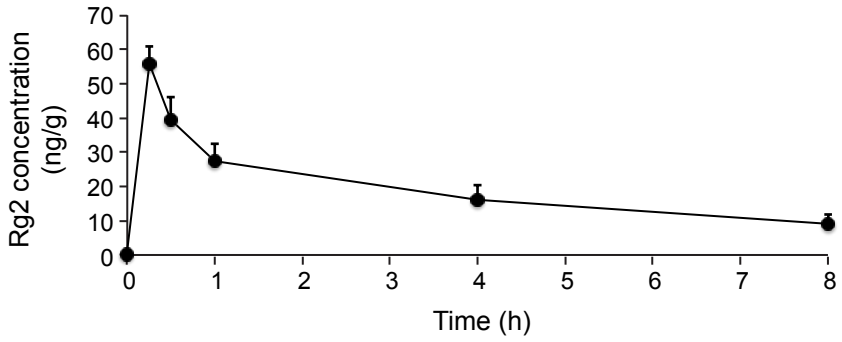
	Ginsenoside Rg2 concentration level (μg/ml)								
	10			50			100		
n	1	2	3	1	2	3	1	2	3
$C_{dialysate}$ (Free)	13.43	13.62	9.32	46.28	49.06	48.72	101.3	99.7	103.4
C_{plasma} (Bound)	22.99	21.46	17.48	60.30	66.67	63.52	120.9	116.9	121.0
Plasma protein binding fraction(%)	41.56	36.51	46.70	23.25	26.42	23.31	16.2	14.7	14.6
Mean binding fraction (Mean ± S.D. %)	41.59± 5.09			24.33 ±1.82			15.2± 0.9		

Supplementary Figure 7

A

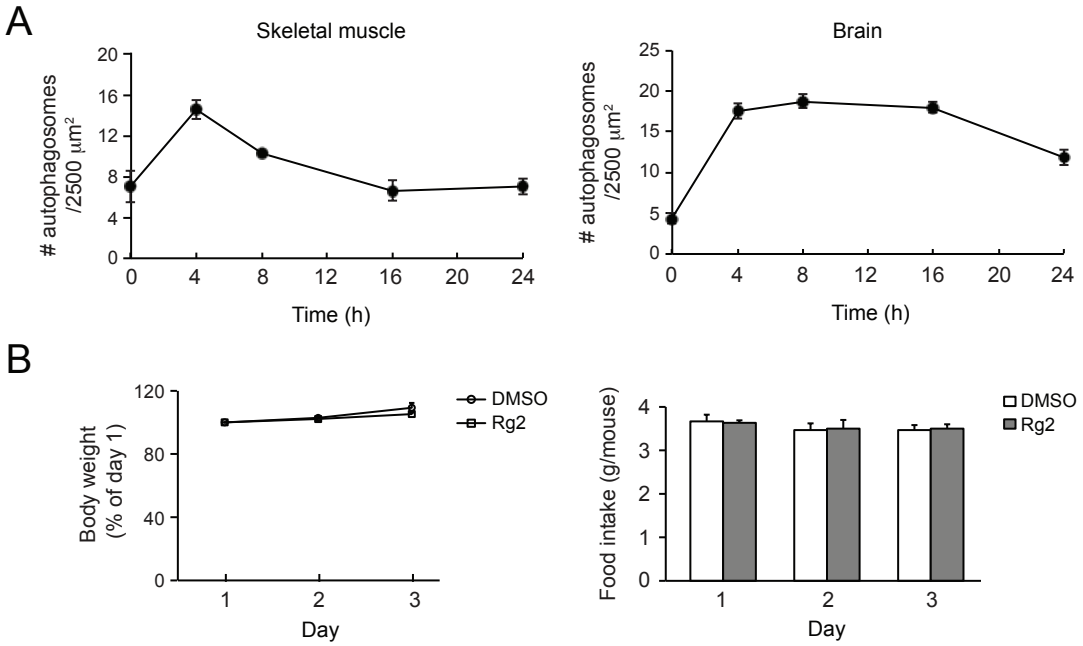


B

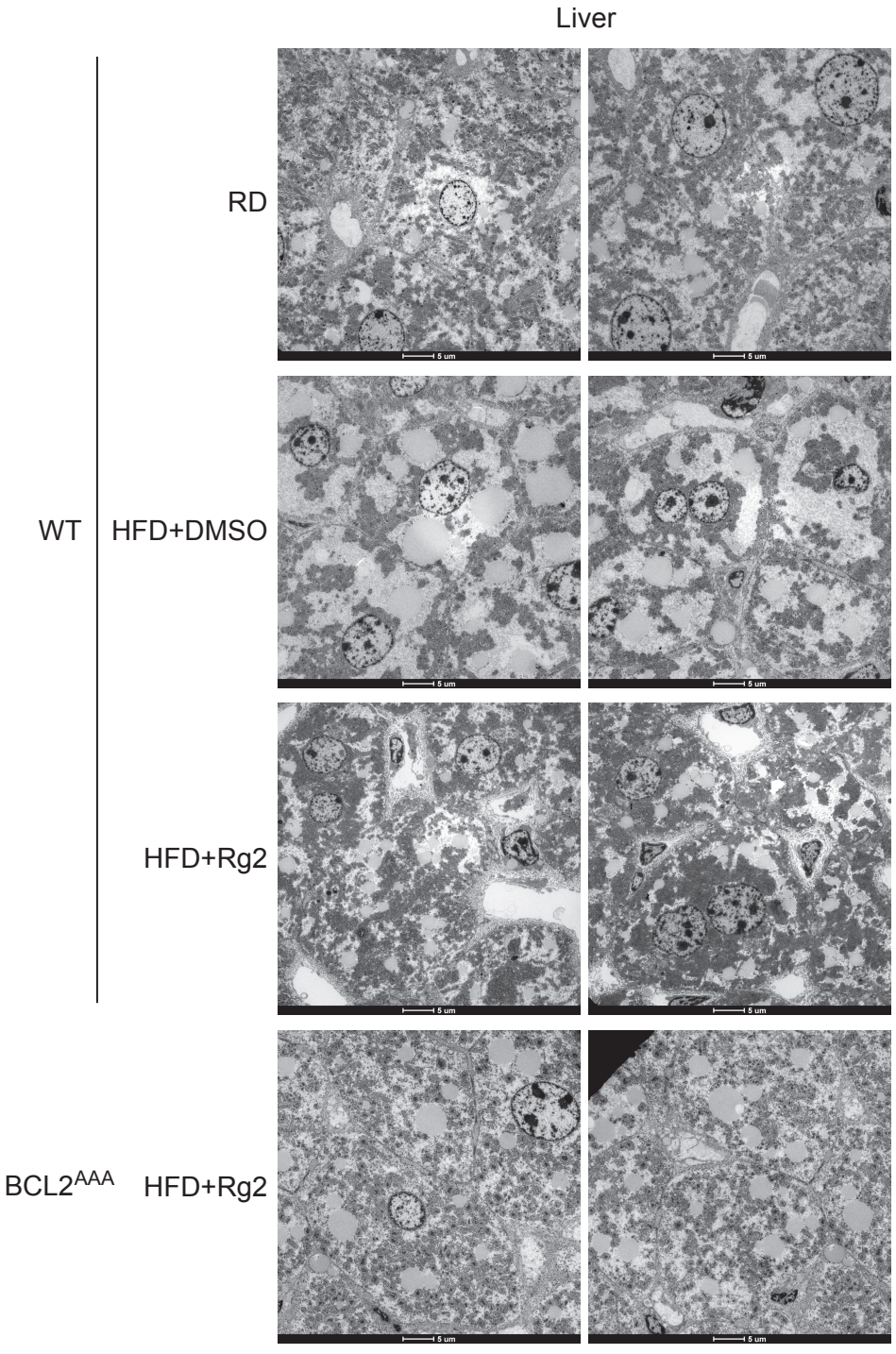


	15 min	30 min	1 h	4 h	8 h	24 h
Concentration (ng/g)	56	39.55	27.225	16.24125	8.94	6.97125
s.e.m. (n=5)	4.796092	6.596732	5.258386	4.188634	2.985672	2.703091

Supplementary Figure 8



Supplementary Figure 9



Supplementary Figure 10